assays. Anti-rPfCDPK-5 antibody levels in the entire cohort shows a positive correlation to morbidity and mortality of children.

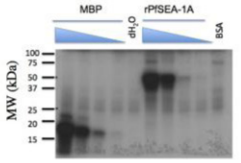

Fig . PfSEA-1A is phosphorylated by rPfCDPK-5 in *in vitro* kinase assay. Positive control substrate (MBP-myelin basic protein), rPfSEA-1A, and negative control substrate (BSA) were incubated with 0.25ug of rPfCDPK5, 1mM $CaCl_2$ and ^{32}P-ATP for 30 min at 37C followed by SDS-PAGE and auto-radiography. Lane 1, 1.0ug of MBP, lane 2, 0.1ug MBP, lane 3, 0.01ug MBP, lane 4, 0.001ug MBP, lane 5, no substrate, lane 6, 1.0ug rPfSEA-1A, lane 7, 0.1ug rPfSEA-1A, lane 8, 0.01ug rPfSEA-1A, lane 9, 0.001ug rPfSEA-1A, lane 10, 1.0ug of BSA. Band at 75 kDa in lanes 6 and 10 represents auto-phosphorylated rPfCDPK5.

PfCDPK-5 phosphorylate PfSEA-1

Conclusion: In the present study, we validate a rationally identified vaccine candidate, *P. falciparum* calcium-dependent protein kinase 5(PfCDPK-5) using integrated translational approaches that harness high-throughput molecular techniques and *in vitro* functional assays.

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Lesson learned from investigating cluster adverse event following immunization in mass campaign of Japanese Encephalitis vaccine in India

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Background: Vaccine safety is one of the critical parameters for quality assurance in immunization program in all countries including developing country like India as it adds new vaccines to its existing immunization program. Occurrence of adverse events following immunization (AEFI) and spread of unchecked rumors can hamper community confidence in vaccines adversely affecting coverage. Cluster AEFI (2 or more reports occurring together) get heightened attention from media, government and community and can affect performance of immunization program. In commitment to improve vaccine safety, this paper summarizes a report of a cluster investigation for AEFIs (90 reports) following Japanese Encephalitis (JE) vaccine given in a mass campaign in one district (Morigoan of Assam), India in June, 2014.

Methods & Materials: In response to received reports of AEFI cluster over 10 day's period in June 2014, National AEFI surveillance team had investigated the reason for the events in the field by interviewing community and reviewing hospital records. Data of the individual cases was entered in anonymized line list and analyzed by using SPSS vs. 16.

campaign and among these 89 of the cases have reported symptoms of dizziness, tingling and numbness and abnormal movement of limbs. More than two- third of the affected individuals were females having median age of 9 years. Cases recovered without any residual sequel after receiving conservative treatment, reassurance and counselling in hospital.

Conclusion: There is a need of multi-pronged, effective information, education and communication intervention to handle unwanted rumors to ensure vaccine confidence during mass campaign by involving multiple stake holders.

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The seroprevalence of neutralizing antibody against Japanese encephalitis virus in health care workers

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Background: Despite the introduction of inactivated Japanese encephalitis (JE) vaccine since 1990, JE remains an important cause of viral encephalitis in Thailand. Little is known about JE serostatus among Thais who were born before the 2001, the year that this vaccine has been included in the expanded immunization program. Therefore, the objective of this study was to determine the proportion of healthcare workers (HCWs) aged 21-60 years with adequate neutralizing antibody against JE virus.

Methods & Materials: We conducted a seroprevalence survey among HCWs during the routine annual check-up for HCWs at Queen Sirikit National Institute of Child Health (Children's Hospital, Bangkok, Thailand) during the period between July-October 2015. A purposive sampling was done to enroll a relatively equal number of 4 different age ranges i.e., 21-30, 31-40, 41-50, and 51-60 years per each group. JE serostatus was determined using 50% Plaque Reduction Neutralization Test (PRNT). Immunity to JE were quantitated and cross-tabulated against age, gender, past and present domicile, history of JE vaccination, types of vaccine received (if any).

Results: A total of 400 HCWs among a total of 1,320 who received annual check-up were enrolled. Only 1.5% of participants reported having immunized with JE vaccine. 80.5% demonstrated an adequate existing antibody against JE virus genotype 3 Beijing strain (at least 10 reciprocal PRNT titer). The proportion of protective antibody (and corresponding geometric mean titer) of the 4 age groups were 77.0% (112.48), 82.4% (211.47), 80.6% (84.59), and 82.0%(126.97) among those aged 21-30, 31-40, 41-50, and 51-60, respectively. Male gender was the only parameter that significantly associated with the lack of protective JE antibody with risk ratio and 95% confidence interval of 1.69 (1.1, 2.7).

Conclusion: Since JE virus is unlikely to be transmitted in hospital settings, the result from this group might be reflective of those in the general adult population in Bangkok. As a result, approximately

20% of adult population in Bangkok may be at risk for acquiring JE when traveling to high risk/endemic area e.g. rural or upcountry.

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Immunogenecity of a chimeric protein of Bacillus anthracis protective antigen and lethal factor in murine model

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Background: Anthrax, a disease of bioterrorism and public health importance is caused by the Gram positive, spore-forming bacterium, *Bacillus anthracis*. Anthrax toxin, a tripartite toxin is composed of protective antigen (PA), lethal factor (LF) or edema factor (EF). PA is the major protein which facilitates the entry of toxin component either of lethal factor or edema factor. Recombinant PA has been a suitable target for anthrax vaccine worldwide. However, instead of full PA, its domains are reported to provide protection. LF also contributes to immuno-protection against anthrax. Therefore, in this study, a chimeric protein consisting of both, PA and LF was developed as candidate vaccine for anthrax.

Methods & Materials: A chimera was made by fusion of immunodominant portion of PA (Domains 2-4) and LF (Domain 1) genes. . The construct was cloned in *pET32a+* vector and expressed in *E. coli* host. The recombinant chimeric protein was purified by immobilized metal affinity chromatography. The 4-6 week old Balb/c mice were injected intraperitonealy with three doses of chimeric protein (20 μg each mouse) at two week interval. The first dose was given with Freund's complete adjuvant and the subesequent doses were given with incomplete Freund's adjuvant. The serum IgG and its subtypes were determined by plate ELISA.

Results: The chimeric protein (PA-LF) was purified up to homogeneity and the production yield was 15 mg/l of the shake flask culture. The chimera elicited good immune response against both the toxins i.e. PA as well as LF. The end point titre of chimeric protein was 1:1024000 by plate ELISA. An antibody titre of 1:512000 was observed in mice serum for PA protein. The same serum exhibited the titre of 1:256000 against LF protein. The end point titres of IgG1, IgG2a, IgG2b and IgG3 were 1: 512000, 1:128000, 1:256000 and 1:32000, respectively. Thus, IgG1 was predominant among all subtypes indicating that PA-LF chimera induced Th2-type immune response

Conclusion: The chimera consisting of partial sequences of PA and LF can be better vaccine candidate than individual PA or LF proteins. In the present study, the recombinant protein elicited very good immune response in mice and showed Th2 type of immune response.

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Antibody response to various domain of protective antigen in cutaneous anthrax cases in India

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Background: Anthrax, caused by *Bacillus anthracis* is a well known biothreat disease. Besides, cutaneous anthrax is a public health disease also several countries where agriculture is the major source of income. Being a zoonotic disease, it primarily infects herbivorous livestock and wildlife species and then spreads to human through contact with infected animals or contaminated animal products. The virulence of *B. anthracis* is attributed to two major factors, i.e. a tripartite toxin and the poly-g-D-glutamic acid capsule. The anthrax toxins are secreted as three distinct proteins, namely protective antigen (PA), lethal factor (LF) and edema factor (EF) and their activities have been well described. PA is the pivotal protein of the anthrax toxin complex and therefore, has been a major target for vaccine development.

Methods & Materials: PA is a 83 kD protein which has 4 different domains. In this study, the 3 different domains of PA were cloned and expressed. The recombinant proteins were used to develop ELISA to determine the anti-PA IgG for individual domain in human cutaneous anthrax serum samples. End-point titers were defined as the highest serum dilutions that yielded an OD_{450nm} value 2-fold the value for the corresponding dilution of the control serum.

Results: Full PA protein (83 kD) and different domain proteins (PAD1, 46 kD; PAD2, 43 kD and PAD4, 33 kD) were purified to the homogeneity. A total of 41 cutaneous anthrax serum samples were examined for immuno-reactivity with PA protein and its domains. The whole PA protein was found to give maximum immunoreactivity followed by domain 4, 2 and 1.

Conclusion: The immunoreactivity of human cutaneous serum samples with individual PA domains showed that besides full PA protein, individual domain 4 and 2 can also be good targets for vaccine development as well as for serodiagnostic assays.

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